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Patentanmeldung Nr. Patent application No. Demande de brevet n°

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Organic compounds

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Organic Compounds

The instant invention relates generally to cathepsin K and their use in bone growth. Specifically, the invention relates to the use of cathepsin K to stimulate new bone formation in patient in need thereof.

Cathepsin K was cloned and found specifically expressed in osteoclasts (Tezuka, K. et al., 1994, J Biol Chem 269:1106-1109; Shi, G. P. et al., 1995, FEBS Lett 357:129-134; Bromme, D. and Okamoto, K., 1995, Biol Chem Hoppe Seyler 376:379-384; Bromme, D. et al., 1996, J Biol Chem 271:2126-2132; Drake, F. H. et al., 1996, J Biol Chem 271: 12511- 12516). Concurrent to the cloning, the autosomal recessive disorder, pycnodysostosis, characterized by an osteopetrosis phenotype with a decrease in bone resorption, was mapped to mutations present in the cathepsin K gene. To date, all mutations identified in the cathepsin K gene are known to result in inactive protein (Gelb, B. D. et al., 1996, Science 273:1236- 1213) & Johnson, M. R. et al., 1996, Genome Res 6:1050-1055. Cathepsin K is synthesized as a '37 kDa pre-pro enzyme, which is localized to the lysosomal compartment and where it is presumably autoactivated to the mature 27 kDa enzyme at low pH (McQueney, M. S. et al., 1997, J Biol Chem 272:13955-13960; Littlewood-Evans, A. et al., 1997, Bone 20:81-86). Cathepsin K is most closely related to cathepsin S having 56 % sequence identity at the amino acid level. The S2P2 substrate specificity of cathepsin K is similar to that of cathepsin S with a preference in the P1 and P2 positions for a positively charged residue such as arginine, and a hydrophobic residue such as phenylalanine or leucine, respectively (Bromme, D. et al., 1996, J Biol Chem 271: 2126-2132; Bossard, M. J. et al., 1996) J Biol Chem 271:12517-12524). Cathepsin K is active at a broad pH range with significant activity between pH 4-8, thus allowing for good catalytic activity in the resorption lacunae of osteoclasts where the pH is about 4-5. Human type I collagen, the major collagen in bone is a good substrate for cathepsin K (Kafienah, W., et al., 1998, Biochem J 331:727-732). In vitro experiments using antisense oligonucleotides to cathepsin K, have shown diminished bone resorption in vitro probably due to a reduction in translation of cathepsin K mRNA (Inui, T., et al., 1997, J Biol Chem 272:8109-8112. The crystal structure of cathepsin K has been resolved (McGrath, M. E., et al., 1997, Nat Struct Biol 4:105- 109; Zhao, B., et al., 1997, Nat Struct Biol 4: 109-11) and selective peptide based inhibitors of cathepsin K (Bromme, D., et al., 1996, Biochem J 315:85-

89-, Thompson, S. K., et al., 1997, Proc Natl Acad Sci U S A 94:14249-14254) and non-peptide

Bone resorption, is primarily performed by multi nuclear giant cells, the osteoclasts. The mechanism by which osteoclasts resorb bone is by an initial cellular attachment to bone tissue followed by the formation of an extracellular compartment or lacunae. The lacunae are maintained at a low pH by a proton-ATP pump. The acidified environment allows for initial demineralization of bone followed by the degradation of bone proteins or collagen by proteases such as cysteine proteases (Delaisse, J. M. et al., 1980, Biochem J 192:365-368; Delaisse, J. et al., 1984, Biochem Biophys Res Commun:441-447; Delaisse, J. M. et al., 1987, Bone 8:305- 313). Collagen constitutes 95 % of the organic matrix of bone. Therefore, proteases such as cathepsin K involved in collagen degradation are an essential component of bone turnover. The skeleton is constantly being remodeled by a balance between osteoblasts that lay down new bone and osteoclasts that breakdown, or resorb bone. In some disease conditions and advancing age the balance between bone formation and resorption is disrupted; bone is removed at a faster rate. Such a prolonged imbalance of resorption over a long duration leads to weaker bone structure and a higher risk of fractures.

In accordance with the present invention, it has now surprisingly been found that cathepsin K inhibitors exert profound and apparently direct bone forming effects in an in vivo animal model. For example, a bone forming effect on certain bones, e.g. measured on bone mineral density (BMD), bone structure, and bone strength is observed when a cathepsin K inhibitor is administered orally to ovariectomized (OVX) cynomolgus monkeys twice daily for eighteen months.

Thus, cathepsin K inhibitors are particularly useful in the treatment of a severe form of various bone loss disorders, including e.g. osteoporosis, osteopenia, Paget's disease of bone, osteogenesis imperfecta (OI), inflammation, rheumatoid arthritis (RA), osteoarthritis (OA), tumors (especially tumor invasion and bone metastases (BM)), tumour-induced hypercalcemia (TH), coronary disease, atherosclerosis (including atherosclerotic plaque rupture and destabilization), autoimmune diseases, respiratory diseases, infectious diseases, immunologically mediated diseases (including transplant rejection) and multiple myeloma (MM).

Accordingly the present invention provides a method for the treatment of a severe form of bone loss diseases in a patient in need of such treatment, which comprises administering an effective amount of a cathepsin K inhibitor to the patient.

The invention further provides the use of a cathepsin K inhibitor in the preparation of a medicament for the treatment of a severe form of bone loss diseases.

The invention yet further provides the use of a cathepsin K inhibitor and other agents useful in the treatment of bone loss diseases to treat a severe form of bone loss diseases in mammals.

Preferably the invention is used for the treatment of diseases and medical conditions in which cathepsin K inhibitors are used to stimulate bone growth. For example, the invention may be used for the treatment of diseases and conditions which involve excessive or inappropriate bone loss e.g. as the result of inappropriate bone metabolism. Examples of such diseases and conditions include severe forms of benign diseases and conditions such as osteoporosis of various genesis, Pagets disease, OA, RA, OI, periodontal disease; and especially malignant diseases such as MM and TIH and BM associated with various cancers, e.g. cancer of the breast, prostate, lung, kidney, ovary, or osteosarcoma. Generally the invention may be used to treat severe bone loss diseases also in other circumstances where cathepsin K inhibitors may be used, e.g. when cathepsin K inhibitors are used in bone fracture healing, osteonecrosis or treatment of prosthesis loosening. Cathepsin K inhibitors are particularly useful for treating severe forms of diseases of bone metabolism including osteoarthritis, osteoporosis and other inflammatory arthritides, and bone loss in general, including age-related bone loss, and in particular periodontal disease.

Furthermore, cathepsin K inhibitors surprisingly improve stiffness, bone quality and/or strength due not only through its anti-resorptive effect (which is expected and known from the literature) but also through its surprising bone-forming effect. Preferably the stiffness, bone quality and strength of osteoporotic bones at the subperiosteal site of the vertebrae and long bones are improved.

Thus, the invention relates to the use of cathepsin K inhibitors for the manufacture of a medicament for reducing the risk of bone fracture in a long bone in a post menopausal woman at risk of or having osteoporosis. The medicament can be employed to increase stiffness and/or toughness at a site of a potential trauma or at a site of an actual trauma. Trauma generally includes fracture, surgical trauma, joint replacement, orthopaedic procedures, and the like. Increasing bone toughness and/or stiffness generally includes increasing mineral density of particular bones, increasing strength of bone, increasing resistance to loading, and the like. Reducing incidence of fracture generally includes reducing the likelihood or actual incidence of fracture for a subject compared to an untreated control population.

As used therein, stiffness refers to the slope of the linear portion of a load-deforming curve and toughness refers to resistance to fracture per unit volume. Each of these can be determined by methods known in the art (e.g. as described in Turner CH, Burr DB 1993 "basic biomechanical measurements of bone: a tutorial." Bone 14: 595-608). Biomechanical properties as known in the art can also be determined e.g. as described in Example 1.

The use of the invention may reduce the risk of trauma or aids recovery from trauma by increasing bone toughness, stiffness or both. Toughness or stiffness of bone results from mass and strength of the bones, e.g. the vertebrae and long bones. The use of the invention can provide levels of bone toughness, stiffness, mass and/or strength within or above the range of the normal population. Preferably the invention provides increased levels relative to the levels resulting from trauma or giving rise to risk of trauma. Increasing toughness, stiffness, or both decreases risk or probability of fracture compared to an untreated control population.

Certain characteristics of bone when increased provide increased bone toughness and/or stiffness. Such characteristics include bone mineral density (BMD), bone mineral content (BMC), bone forming marker, bone formation, cortical porosity, cross sectional bone area and bone mass, resistance to loading, and/or work to failure (for definitions see Turner, supra). An increase in one or more of these characteristics is a preferred outcome of the invention.

The invention may be effective for increasing the toughness and/or stiffness of any of several bones. For example, the present invention can increase the toughness and/or stiffness of bones including e.g. vertebrae and/or long bones.

Thus in the present description the terms "treatment" or "treat" refer to both prophylactic or preventative treatment as well as curative or direct treatment of severe bone loss diseases, in particular treatment of severe osteoporosis.

Thus in particular embodiments the invention provides: a method for the treatment of a severe form of bone loss diseases in a patient in need of such treatment which comprises administering an effective amount of a cathepsin K inhibitor to the patient; the use of a cathepsin K inhibitor in the preparation of a medicament for the treatment of a severe form or severe forms of bone loss diseases; or the use of a cathepsin K inhibitor as an agent for treatment of a severe form or severe forms of bone loss diseases.

For these indications, the appropriate dosage will, of course, vary depending upon, for example, the particular cathepsin K inhibitor to be employed, the host, the mode of administration and the nature and severity of the condition being treated. However, in general, satisfactory results in animals are indicated to be obtained at a daily dosage from about 1 to about 300 mg/kg animal body weight. In larger mammals, for example humans, an indicated daily dosage is in the range from about 0.1 to about 2 g of a compound according to the invention, conveniently administered, for example, in divided doses up to four times a day. The cathepsin K inhibitors may be administered in any usual manner, e.g. orally, for example in the form of tablets or capsules, or parenterally, for example in the form of injection solutions or solutions.

The present invention also provides pharmaceutical compositions comprising the cathepsin K inhibitors in association with at least one pharmaceutical carrier or diluent for use in the treatment of a severe form of bone loss diseases. Such compositions may be manufactured in conventional manner. Unit dosage forms may contain for example from about 2.5mg to about 1000 mg of the cathepsin K inhibitor.

The cathepsin K inhibitors may be administered as the sole active ingredient or in conjunction with, e.g. as an adjuvant to, another therapeutic agent. Examples of other therapeutic agents include, but are not limited to, agents useful for treating or preventing a bone-resorbing disease, a neoplastic disease, arthritis, a disease exacerbated by the presence of a cathepsin K inhibitor or a disease improved by the presence of a cathepsin K inhibitor; activating the function of cathepsin K in a bone cell; inhibiting the function of cathepsin K in a cancer cell; inhibiting the expression of cathepsin K in a cell; and inhibiting the growth of a neoplastic cell. The other therapeutic agent can be administered before, after or concurrently with the cathepsin K inhibitors. In these embodiments, the time at which the cathepsin K inhibitors exerts its therapeutic effect on the patient overlaps with the time at which the other therapeutic agent exerts its therapeutic effect on the patient.

In one embodiment, the other therapeutic agent is useful for the treatment or prevention of a bone-loss disease (e.g., osteoporosis). Other therapeutic agents useful for the treatment or prevention of a bone-loss disease include, but are not limited to, other cathepsin K inhibitors than the first cathepsin K inhibitor (e.g., a pro-peptide of cathepsin K), bisphosphonates (e.g., etidronate, pamidronate, alendronate, risedronate, zolendronate, ibandronate, clodronate or tiludronate), Selective Estrogen Receptor Modulators (SERMs), such as tamoxifen, raloxifene, medroxyprogesterone, danazol and gestrinone, parathyroid hormone ("PTH") or fragments thereof, compounds that release endogenous PTH (e.g., a PTH releasing hormone) and calcitonin or fragments thereof.

In another embodiment, the other therapeutic agent is useful for the treatment or prevention of a neoplastic disease. In one embodiment, the other therapeutic agent is useful for the treatment or prevention of cancer (e.g., cancer of the breast, ovary, uterine, prostate or hypothalamus). Other therapeutic agents useful for the treatment or prevention of cancer or a neoplastic disease include, but are not limited to, alkylating agents (e.g., nitrosoureas), an anti-metabolite (e.g., methotrexate or hydroxyurea), etoposides, camptothecins, bleomycin, doxorubicin, daunorubicin, colchicine, irinotecan, camptothecin, cyclophosphamide, 5-fluorouracil, cisplatin, carboplatin, methotrexate, trimetrexate, erbitux, thalidomide, taxol, a-vinca alkaloid (e.g., vinblastine or vincristine) or a microtubule stabilizer (e.g., an epothilone).

Further illustrative examples of therapeutic agents useful for the treatment or prevention of cancer include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; balmastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crismatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitucin; enoplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofosine; ImiDs; interleukin II (including recombinant interleukin II, or rIL2), interferon -2a; interferon alpha-2b; interferon alpha-n1; interferon alpha-n3; interferon beta-1 a; interferon gamma-1 b; iproplatin; irinotecan hydrochloride; laurootide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptapurine; methotrexate; methotrexate sodium; metoprime; meturedopa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; pipoisulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; SelCid; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprime; thioguanine;

temozolomide; temodar; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride.

Other therapeutic agents useful for the treatment or prevention of cancer include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecyepenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzopyranones, benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); cell-cycle inhibitors (e.g., flavopiridol A, tryprostatin B, p19ink4D); cyclin-dependent kinase inhibitors (e.g., roscovitine, olomucine and purine analogs); MAP kinase inhibitors (CNI-1493); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; daclizimab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene;

dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+mycobacterium cell wall sk; molidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisarnycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxazomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil;

pilocarpine hydrochloride; pirarubicin; piritrexim; placeti A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; retinoic acid (e.g., 9-cis RA); histone deacetylase inhibitors (e.g., sodium butyrate, suberoylanilide hydroxamic acid); TRAIL; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogliptide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; typhostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer. Preferred additional anti-cancer drugs are 5-fluorouracil and leucovorin.

In accordance with the foregoing the present invention provides in a yet further aspect:

4-33509P1

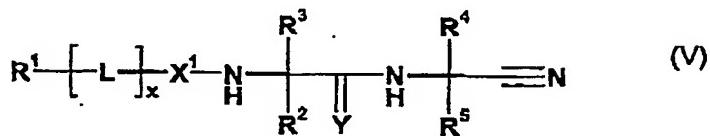
A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of a cathepsin K inhibitor, and at least one second drug substance, said second drug substance being a therapeutic agent against bone loss diseases, e.g. as indicated above.

Or, a therapeutic combination, e.g. a kit, comprising of a therapeutically effective amount of a) a cathepsin K inhibitor, and b) at least one second substance selected from a therapeutic agent against bone loss diseases, e.g. as indicated above. The kit may comprise instructions for its administration.

Where the cathepsin K inhibitors are administered in conjunction with other therapeutic agents against bone loss diseases, dosages of the co-administered combination compound will of course vary depending on the type of co-drug employed, e.g. whether it is a bisphosphonate, a SERMs, a calcitonin, a PTH, a PTH fragment or a PTH analogue or others, on the specific drug employed, on the condition being treated and so forth. Pharmaceutical compositions comprising cathepsin K inhibitors and a second drug substance may be manufactured in conventional manner. A composition according to the invention is preferably provided in tablet form.

The cathepsin K inhibitors used in the present invention are typically those which form bone, in particular stimulate cortical bone formation at subperiosteal site, i.e. vertebrae and long bones. Preferably, the cat K inhibitors used in the pharmaceutical compositions and treatment methods of the present invention typically comprises a cathepsin K inhibitor, e.g. disclosed in WO 9523222, WO 9630353, WO 9640737, WO 9716433, WO 9801133, WO 9805336, WO 9808802, WO 9846582, WO 9848799, WO 9849152, WO 9850342, WO 9850533, WO 9850534, WO 9911637, WO 9924460, WO 9948911, WO 9959526, WO 9959570, WO 9964399, WO 9966925, WO 0029408, WO 0038687, WO 0039115, WO 0048993, WO 0049011, WO 0054769, WO 0055124, WO 0055125, WO 0055126, WO 0055144, WO 0058296, WO 0059881, WO 0109110, WO 0109169, WO 0119808, WO 0119816, WO 0134153, WO 0134154, WO 0134155, WO 0134156, WO 0134157, WO 0134158, WO 0320721, WO 0320278, WO 0313518, WO 02100849, WO 0298406, WO 0296892, WO 0292563, WO 0288106, WO 0280920, WO 0270519, WO 0270517, WO 0269992, WO 0269901, WO 0257270, WO 0257249, WO 0257248, WO 0257246, WO 0158886, WO 0155123

or a compound of formula V, or a physiologically acceptable and -cleavable ester or a salt thereof



wherein  $\text{R}^1$  is optionally substituted (aryl, aryl-lower alkyl, lower alkenyl, lower alkynyl, heterocyclyl or heterocyclyl-lower alkyl);

$\text{R}^2$  and  $\text{R}^3$  together represent lower alkylene, optionally interrupted by O, S or  $\text{NR}^6$ , so as to form a ring with the carbon atom to which they are attached, and  $\text{R}^6$  is hydrogen, lower alkyl or aryl-lower alkyl;

$\text{R}^4$  and  $\text{R}^5$  are independently H, or optionally substituted (lower alkyl or aryl-lower alkyl),  $-\text{C}(\text{O})\text{OR}^7$ , or  $-\text{C}(\text{O})\text{NR}^7\text{R}^8$ , wherein  $\text{R}^7$  is optionally substituted (lower alkyl, aryl, aryl-lower alkyl, cycloalkyl, bicycloalkyl, bicycloalkyl or heterocyclyl), and  $\text{R}^8$  is H, or optionally substituted (lower alkyl, aryl, aryl-lower alkyl, cycloalkyl, bicycloalkyl, bicycloalkyl or heterocyclyl); or  $\text{R}^4$  and  $\text{R}^5$  together represent lower alkylene, optionally interrupted by O, S or  $\text{NR}^6$ , so as to form a ring with the carbon atom to which they are attached, and  $\text{R}^6$  is hydrogen, lower alkyl or aryl-lower alkyl; or

$\text{R}^4$  is H or optionally substituted lower alkyl and  $\text{R}^5$  is a substituent of formula  $-\text{X}^2-(\text{Y}^1)_n-(\text{Ar})_p-\text{Q}-\text{Z}$  wherein

$\text{Y}^1$  is O, S, SO,  $\text{SO}_2$ ,  $\text{N}(\text{R}^6)\text{SO}_2$ ,  $\text{N}-\text{R}^6$ ,  $\text{SO}_2\text{NR}^6$ ,  $\text{CONR}^6$  or  $\text{NR}^6\text{CO}$ ;

N is zero or one;

P is zero or one;

$\text{X}^2$  is lower alkylene; or when n is zero,  $\text{X}^2$  is also  $\text{C}_2\text{-C}_7$ -alkylene interrupted by O, S, SO,  $\text{SO}_2$ ,  $\text{NR}^6$ ,  $\text{SO}_2\text{NR}^6$ ,  $\text{CONR}^6$  or  $\text{NR}^6\text{CO}$ , and  $\text{R}^6$  is hydrogen, lower alkyl or aryl-lower alkyl;

Ar is arylene;

Z is hydroxyl, acyloxy, carboxyl, esterified carboxyl, amidated carboxyl, aminosulfonyl, (lower alkyl or aryl-lower alkyl)aminosulfonyl, or (lower alkyl or aryl-lower alkyl)sulfonylaminocarbonyl; or Z is tetrazolyl, triazolyl or imidazolyl;

Q is a direct bond, lower alkylene,  $\text{Y}^1$ -lower alkylene or  $\text{C}_2\text{-C}_7$ -alkylene interrupted by  $\text{Y}^1$ ;

$\text{X}^1$  is  $-\text{C}(\text{O})-$ ,  $-\text{C}(\text{S})-$ ,  $-\text{S}(\text{O})-$ ,  $-\text{S}(\text{O})_2-$ , or  $-\text{P}(\text{O})(\text{OR}^6)-$ , and  $\text{R}^6$  is as defined above;

Y is oxygen or sulphur;

L is optionally substituted -Het-, -Het-CH<sub>2</sub>- or -CH<sub>2</sub>-Het-, and Het is a hetero atom selected from O, N or S; and

X is zero or one; and

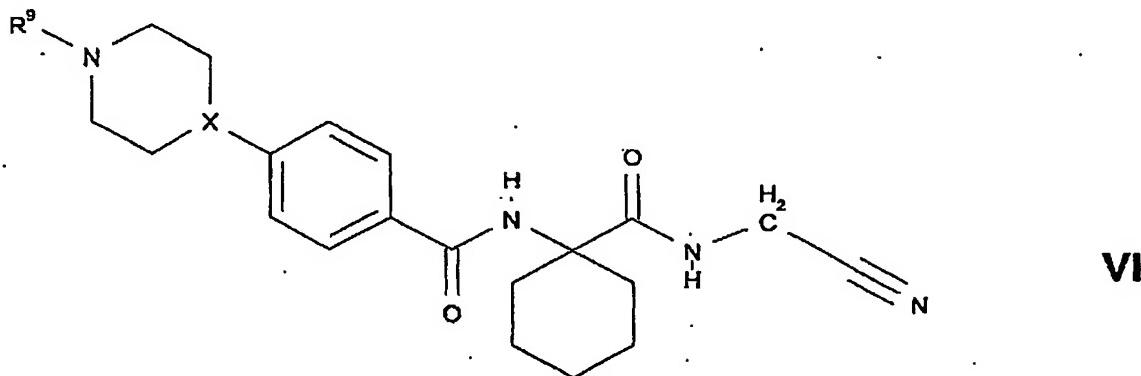
aryl in the above definitions represents carbocyclic or heterocyclic aryl.

Particular compounds of formula V are those wherein R<sup>1</sup> is a substituted phenyl, e.g. whereas the substituent is an optionally substituted nitrogen-containing heterocyclic substituent (=Het<sup>IV</sup>). This substituent may be at the 2- or 3- position of the phenyl ring, though preferably at the 4-position. Het<sup>IV</sup> signifies a heterocyclic ring system containing at least one nitrogen atom, from 2 to 10, preferably from 3 to 7, most preferably 4 or 5, carbon atoms and optionally one or more additional heteroatoms selected from O, S or preferably N.

Het<sup>IV</sup> may comprise an unsaturated, e.g. an aromatic, nitrogen-containing heterocycle; though preferably comprises a saturated nitrogen-containing heterocycle. Particularly preferred saturated nitrogen-containing heterocycles are piperazinyl, preferably piperazin-1-yl, or piperidinyl, preferably piperidin-4-yl.

Het<sup>IV</sup> may be substituted by one or more substituents, e.g. by up to 5 substituents independently selected from halogen, hydroxy, amino, nitro, optionally substituted C<sub>1</sub>-alkyl (e.g. alkyl substituted by hydroxy, alkyloxy, amino, optionally substituted alkylamino, optionally substituted dialkylamino, aryl or heterocycl), C<sub>1</sub>-alkoxy. Preferably Het<sup>IV</sup> is substituted at a nitrogen atom, most preferably mono-substituted at a nitrogen atom. Preferred substituents for Het<sup>IV</sup> are C<sub>1</sub>-C<sub>7</sub>lower alkyl, C<sub>1</sub>-C<sub>7</sub>lower alkoxy-C<sub>1</sub>-C<sub>7</sub>lower alkyl, C<sub>5</sub>-C<sub>10</sub>aryl-C<sub>1</sub>-C<sub>7</sub>lower alkyl, or C<sub>3</sub>-C<sub>8</sub>cycloalkyl.

Particularly preferred embodiments of the invention provides a compound of formula VI, or a pharmaceutically acceptable salt or ester thereof:



wherein X is CH or N, and

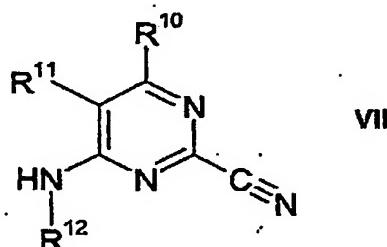
$R^9$  is H, C<sub>1</sub>-C<sub>7</sub>lower alkyl, C<sub>1</sub>-C<sub>7</sub>lower alkoxy-C<sub>1</sub>-C<sub>7</sub>lower alkyl, C<sub>5</sub>-C<sub>10</sub>aryl-C<sub>1</sub>-C<sub>7</sub>lower alkyl, or C<sub>3</sub>-C<sub>8</sub>cycloalkyl.

Thus particular examples of  $R^9$  as C<sub>1</sub>-C<sub>7</sub>lower alkyl are methyl, ethyl, n-propyl, or i-propyl are preferred. A particular example of R as C<sub>1</sub>-C<sub>7</sub>lower alkoxy-C<sub>1</sub>-C<sub>7</sub>lower alkyl is methoxyethyl. A particular example of R as C<sub>5</sub>-C<sub>10</sub>aryl-C<sub>1</sub>-C<sub>7</sub>lower alkyl is benzyl. A particular example of R as C<sub>3</sub>-C<sub>8</sub>cycloalkyl is cyclopentyl. Examples of particular compounds of formula VI are: N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(piperazin-1-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(4-methyl-piperazin-1-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(4-ethyl-piperazin-1-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-[4-(1-propyl)-piperazin-1-yl]-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(4-isopropyl-piperazin-1-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(4-benzyl-piperazin-1-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-[4-(2-methoxy-ethyl)-piperazin-1-yl]-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(1-propyl-piperidin-4-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-[1-(2-methoxy-ethyl)-piperidin-4-yl]-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(1-isopropyl-piperidin-4-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(1-cyclopentyl-piperidin-4-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(1-methyl-piperidin-4-yl)-benzamide, and N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(piperidin-4-yl)-benzamide.

The most preferred cat K inhibitor for use in the invention is N-[1-(Cyanomethylcarbamoyl)-cyclohexyl]-4-[4-(1-propyl)-piperazin-1-yl]-benzamide or a pharmacologically acceptable salt thereof.

All the cat K inhibitors mentioned above are known from the literature. This includes their production (see e.g. US 6,353,017B1, pp. 15-17).

An alternative class of cat K inhibitors compounds for use in the invention comprises a compound of formula VII, or a physiologically acceptable and -cleavable ester or a salt thereof



wherein

R<sup>10</sup> is H, -R<sup>14</sup>, -OR<sup>14</sup> or NR<sup>13</sup>R<sup>14</sup>,

wherein R<sup>13</sup> is H, lower alkyl or C<sub>3</sub> to C<sub>10</sub> cycloalkyl, and

R<sup>14</sup> is lower alkyl or C<sub>3</sub> to C<sub>10</sub> cycloalkyl, and

wherein R<sup>13</sup> and R<sup>14</sup> are independently, optionally substituted by halo, hydroxy, lower alkoxy, CN, NO<sub>2</sub>, or optionally mono- or di-lower alkyl substituted amino;

R<sup>11</sup> is -CO-NR<sup>15</sup>R<sup>16</sup>, -NH-CO-R<sup>15</sup>, -CH<sub>2</sub>-NH-C(O)-R<sup>15</sup>, -CO-R<sup>15</sup>, -S(O)-R<sup>15</sup>, -S(O)<sub>2</sub>-R<sup>15</sup>, -CH<sub>2</sub>-CO-R<sup>15</sup> or -CH<sub>2</sub>-NR<sup>15</sup>R<sup>16</sup>,

wherein

R<sup>15</sup> is aryl, aryl-lower alkyl, C<sub>3</sub>-C<sub>10</sub>cycloalkyl, C<sub>3</sub>-C<sub>10</sub>cycloalkyl-lower alkyl, heterocyclyl or heterocyclyl-lower alkyl,

R<sup>16</sup> is H, aryl, aryl-lower alkyl, aryl-lower-alkenyl, C<sub>3</sub>-C<sub>10</sub>cycloalkyl, C<sub>3</sub>-C<sub>10</sub>cycloalkyl-lower alkyl, heterocyclyl or heterocyclyl-lower alkyl, or

wherein R<sup>15</sup> and R<sup>16</sup> together with the nitrogen atom to which they attached are joined to form an N-heterocyclyl group,

wherein N-heterocyclyl denotes a saturated, partially unsaturated or aromatic nitrogen containing

heterocyclic moiety attached via a nitrogen atom thereof having from 3 to 8 ring atoms optionally substituted by one or more groups selected from halo, hydroxy, oxo, lower alkoxy, CN or NO<sub>2</sub>, or optionally substituted (lower alkyl, carboxy, acyl (including both lower alkyl acyl, e.g. formyl, acetyl or propionyl, or aryl acyl, e.g. benzoyl), amido, aryl, S(O) or S(O)<sub>2</sub>), and wherein the N-heterocyclyl is optionally fused in a bicyclic structure, e.g. with a benzene or pyridine ring, and wherein the N-heterocyclyl is optionally linked in a spiro structure with a 3 to 8 membered cycloalkyl or heterocyclic ring wherein the heterocyclic ring has from 3 to 10 ring members and contains from 1 to 3 heteroatoms selected from N, NR<sup>16</sup>, O, S, S(O) or S(O)<sub>2</sub> wherein R<sup>16</sup> is as defined above), and

wherein heterocyclyl denotes a ring having from 3 to 10 ring members and containing from 1 to 3 heteroatoms selected from N, NR<sup>17</sup>, O, S, S(O) or S(O)<sub>2</sub> wherein R<sup>17</sup> is as defined above), and wherein R<sup>15</sup> and R<sup>16</sup> are independently, optionally substituted by one or more groups, e.g. 1-3 groups, selected from halo, hydroxy, oxo, lower alkoxy, CN or NO<sub>2</sub>, or optionally substituted (optionally mono- or di-lower alkyl substituted amino, lower-alkoxy, aryl, aryl-lower alkyl, N-heterocyclyl or N-heterocyclyl-lower alkyl (wherein the optional substitution comprises from 1 to 3 substituents selected from halo, hydroxy, lower alkoxy, lower alkoxy-lower alkyl, lower alkoxy-carbonyl, CN, NO<sub>2</sub>, N-heterocyclyl or N-heterocyclyl-lower alkyl, or optionally mono- or di-lower alkyl substituted amino;

R<sup>12</sup> is independently H, or optionally substituted (lower alkyl, aryl, aryl-lower alkyl, C<sub>3</sub>-C<sub>10</sub>cycloalkyl, C<sub>3</sub>-C<sub>10</sub>cycloalkyl-lower alkyl, heterocyclyl or heterocyclyl-lower alkyl), and wherein R<sup>2</sup> is optionally substituted by halo, hydroxy, oxo, lower alkoxy, CN, NO<sub>2</sub>, or optionally mono- or di-lower alkyl substituted amino.

Halo or halogen denote I, Br, Cl or F.

The term "lower" referred to above and hereinafter in connection with organic radicals or compounds respectively defines such as branched or unbranched with up to and including 7, preferably up to and including 5 and advantageously one, two or three carbon atoms.

A lower alkyl group is branched or unbranched and contains 1 to 7 carbon atoms, preferably 1-5 carbon atoms. Lower alkyl represents; for example, methyl, ethyl, propyl, butyl, isopropyl isobutyl, tertiary butyl or neopentyl (2,2-dimethylpropyl).

Halo-substituted lower alkyl is C<sub>1</sub>-C<sub>7</sub>lower alkyl substituted by up to 6 halo atoms.

A lower alkoxy group is branched or unbranched and contains 1 to 7 carbon atoms, preferably 1-4 carbon atoms. Lower alkoxy represents for example methoxy, ethoxy, propoxy, butoxy, isopropoxy, isobutoxy or tertiary butoxy.

A lower alkene, alkenyl or alkenyloxy group is branched or unbranched and contains 2 to 7 carbon atoms, preferably 2-4 carbon atoms and contains at least one carbon-carbon double bond. Lower alkene lower alkenyl or lower alkenyloxy represents for example vinyl, prop-1-enyl, allyl, butenyl, isopropenyl or isobutenyl and the oxy equivalents thereof.

A lower alkyne, alkynyl or alkynyloxy group is branched or unbranched and contains 2 to 7 carbon atoms, preferably 2-4 carbon atoms and contains at least one carbon-carbon triple bond. Lower alkyne or alkynyl represents for example ethynyl, prop-1-ynyl, propargyl, butynyl, isopropynyl or isobutynyl and the oxy equivalents thereof.

In the present description, oxygen containing substituents, e.g. alkoxy, alkenyloxy, alkynyloxy, carbonyl, etc. encompass their sulphur containing homologues, e.g. thioalkoxy, thioalkenyloxy, thioalkynyloxy, thiocarbonyl, sulphone, sulphoxide etc.

Aryl represents carbocyclic or heterocyclic aryl.

Carbocyclic aryl represents monocyclic, bicyclic or tricyclic aryl, for example phenyl or phenyl mono-, di- or tri-substituted by one, two or three radicals selected from lower alkyl, lower alkoxy, aryl, hydroxy, halogen, cyano, trifluoromethyl, lower alkyleneedioxy and oxy-C<sub>2</sub>-C<sub>3</sub>-alkylene and other substituents, for instance as described in the examples; or 1- or 2-naphthyl; or 1- or 2-phenanthrenyl. Lower alkyleneedioxy is a divalent substituent attached to two adjacent carbon atoms of phenyl, e.g. methylenedioxy or ethylenedioxy. Oxy-C<sub>2</sub>-C<sub>3</sub>-alkylene is also a divalent substituent attached to two adjacent carbon atoms of phenyl, e.g. oxyethylene or oxypropylene. An example for oxy-C<sub>2</sub>-C<sub>3</sub>-alkylene-phenyl is 2,3-dihydrobenzofuran-5-yl.

Preferred as carbocyclic aryl is naphthyl, phenyl or phenyl optionally substituted, for

instance, as described in the examples, e.g. mono- or disubstituted by lower alkoxy, phenyl, halogen, lower alkyl or trifluoromethyl.

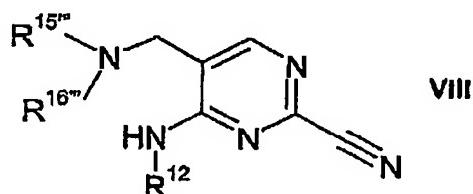
Heterocyclic aryl represents monocyclic or bicyclic heteroaryl, for example pyridyl, indolyl, quinoxalinyl, quinolinyl, isoquinolinyl, benzothienyl, benzofuranyl, benzopyranyl, benzothiopyranyl, furanyl, pyrrolyl, thiazolyl, oxazolyl, isoxazolyl, triazolyl, tetrazolyl, pyrazolyl, imidazolyl, thienyl, or any said radical substituted, especially mono- or di-substituted as defined above.

Preferably, heterocyclic aryl is pyridyl, indolyl, quinolinyl, pyrrolyl, thiazolyl, isoxazolyl, triazolyl, tetrazolyl, pyrazolyl, imidazolyl, thienyl, or any said radical substituted, especially mono- or di-substituted as defined above.

Cycloalkyl represents a saturated cyclic hydrocarbon optionally substituted by lower alkyl which contains 3 to 10 ring carbons and is advantageously cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl or cyclooctyl optionally substituted by lower alkyl.

N-heterocyclyl is as defined above. Preferred N-heterocyclic substituents are optionally substituted pyrrolidine, pyrrole, diazole, triazole, tetrazole, imidazole, oxazole, thiazole, pyridine, pyrimidine, triazine, piperidine, piperazine, morpholine, phthalimide, hydantoin, oxazolidinone or 2,6-dioxo-piperazine and, for example, as hereinafter described in the examples.

In a further embodiment the invention provides a compound of formula VIII, or a pharmaceutically acceptable salt or ester thereof

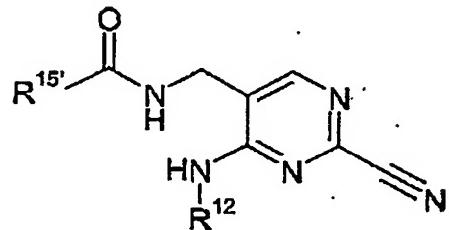


wherein R<sup>12</sup> is as defined above and R<sup>15</sup>, and R<sup>16</sup>, are as defined above for R<sup>15</sup> and R<sup>16</sup> respectively.

$R^{12}$  is preferably  $R^{12}$ , which is lower alkyl, e.g. straight chain or more preferably branched-chain C<sub>1</sub>-C<sub>6</sub> alkyl, e.g. especially 2-ethylbutyl, isobutyl, or 2,2-dimethylpropyl; or C<sub>3</sub>-C<sub>6</sub>cycloalkyl, especially cyclopropyl, cyclopentyl or cyclohexyl.

$R^{15,,}$  and  $R^{16,,}$  may be such that  $R^{15,,}$  and  $R^{16,,}$  together with the nitrogen atom to which they are joined to form an N-heterocyclyl group.  $R^{15,,}$  is preferably optionally substituted (aryl-lower-alkyl, heterocyclyl-aryl, N-heterocyclyl-aryl or aryl-N-heterocyclyl (where N-heterocyclyl is as defined above)).  $R^{15,,}$  is preferably optionally substituted by from 1-4 substituents selected from halo, hydroxy, nitro, cyano, lower-alkyl, lower-alkoxy or lower-alkoxy-lower-alkyl. For example,  $R^{15,,}$  is 4-methoxy-benzyl, 3-methoxy-benzyl, 4-(4-methyl-piperazin-1-yl)-benzyl, 4-[4-(2-ethoxy-ethyl)-piperazin-1-yl]-benzyl, 1-methyl-1-phenyl-ethyl, 2-(4-methoxy-phenyl)-1,1-dimethyl-ethyl, 2-(4-fluoro-phenyl)-1,1-dimethyl-ethyl, 4-(4-methyl-piperazin-1-yl)-phenyl]-ethyl, 2-[4-(4-isopropyl-piperazin-1-yl)-phenyl]-1,1-dimethyl-ethyl, 2-{4-[4-(2-methoxy-ethyl)-piperazin-1-yl]-phenyl}-1,1-dimethyl-ethyl, 2-{3-[4-(2-ethoxy-ethyl)-piperazin-1-yl]-phenyl}-1,1-dimethyl-ethyl, 2-[3-(4-isopropyl-piperazin-1-yl)-phenyl]-1,1-dimethyl-ethyl, 2-(3-pyrrolidin-1-yl-phenyl)-ethyl, 2-{3-[4-(2-methoxy-ethyl)-piperazin-1-yl]-phenyl}-1,1-dimethyl-ethyl, 2-(4-methoxy-phenyl)-ethyl, 2-[4-(4-methyl-piperazin-1-yl)-phenyl]-ethyl, 2-[4-(4-isopropyl-piperazin-1-yl)-phenyl]-ethyl, 2-{4-[4-(2-methoxy-ethyl)-piperazin-1-yl]-phenyl}-ethyl, 2-(3-methoxy-phenyl)-ethyl, 2-[3-(4-methyl-piperazin-1-yl)-phenyl]-ethyl, 2-[4-(4-isopropyl-piperazin-1-yl)-phenyl]-ethyl, 2-pyrrol-1-yl-ethyl, 3-piperidin-1-yl-propyl, 2-(4-methoxy-phenyl)-2-methyl-propyl, 2-methyl-2-[4-(4-methyl-piperazin-1-yl)-phenyl]-propyl, 2-[4-(4-isopropyl-piperazin-1-yl)-phenyl]-2-methyl-propyl, 2-{4-[4-(2-ethoxy-ethyl)-piperazin-1-yl]-phenyl}-2-methyl-propyl, 2-[4-(pyrimidin-1-yl)-phenyl]-2-methyl-propyl, 4-(3-methoxy-phenyl)-piperazin-1-yl-methyl, 4-(4-methoxy-phenyl)-piperazin-1-yl-methyl, 1-methyl-1-(1-phenyl-cyclopropyl)-ethyl. For example,  $R^{15,,}$  and  $R^{16,,}$  together with the nitrogen atom to which they are joined to form an N-heterocyclyl group is 4-(2-pyridin-4-yl-ethyl)-piperazin-1-yl, [4-(2-pyridin-2-yl-ethyl)-piperazin-1-yl, 4-pyridin-4-ylmethyl-piperazin-1-yl, 4-(2-piperidin-1-yl-ethyl)-piperazin-1-yl, 4-(2-pyrrolidin-1-yl-ethyl)-piperazin-1-yl, 4-(2-Diethylamino-ethyl)-piperazin-1-yl, 4-(3-Diethylamino-propyl)-piperazin-1-yl, 4-(1-methyl-piperidin-4-yl)-piperazin-1-yl, 4-pyrrolidin-1-yl-piperidin-1-yl, 4-(2-methoxy-ethyl)-piperazin-1-yl].

In a preferred embodiment the invention provides the use according to the invention of a compound of formula:



wherein R<sup>12</sup> is as defined above and R<sup>15'</sup> is as defined above for R<sup>15</sup>.

R<sup>12</sup> is preferably R<sup>12'</sup>, which is lower alkyl, e.g. straight chain or more preferably branched-chain C<sub>1</sub>-C<sub>6</sub> alkyl, e.g. especially 2-ethylbutyl, isobutyl, or 2,2-dimethylpropyl; or C<sub>3</sub>-C<sub>6</sub>cycloalkyl, especially cyclopropyl, cyclopentyl or cyclohexyl.

R<sup>15'</sup> is preferably optionally substituted (aryl-lower-alkyl, heterocyclyl-aryl, N-heterocyclyl-aryl or aryl-N-heterocyclyl (where N-heterocyclyl is as defined above). R<sup>15'</sup> is preferably optionally substituted by from 1-4 substituents selected from halo, hydroxy, nitro, cyano, lower-alkyl, lower-alkoxy, lower-alkoxy-carbonyl or lower-alkoxy-lower-alkyl. For example, R<sup>15'</sup> is 4-methoxy-phenyl, 4-(1-propyl-piperidin-4-yl)-phenyl, 4-(4-methyl-piperazin-1-yl)-phenyl, 4-[1-(2-methoxy-ethyl)-piperidin-4-yl]-phenyl, 4-(4-propyl-piperazin-1-yl)-phenyl, 3-[4-(4-methyl-piperazin-1-yl)-phenyl]-propionyl, 3-[3-(4-methyl-piperazin-1-yl)-phenyl]-propionyl, 4-(4-ethyl-piperazin-1-yl)-phenyl, 4-(4-isopropyl-piperazin-1-yl)-phenyl, 4-[4-(2-ethoxy-ethyl)-piperazin-1-yl]-phenyl, 4-[4-(2-methoxy-ethyl)-piperazin-1-yl]-phenyl, 4-piperazin-1-yl-phenyl, 4-[4-(carboxylic acid tert-butyl ester) piperazino-1-yl]-phenyl, 3-[4-(carboxylic acid tert-butyl ester) piperazino-1-yl]-phenyl, 3-(4-methyl-piperazin-1-yl)-phenyl, 3-(4-ethyl-piperazin-1-yl)-phenyl, 3-(4-isopropyl-piperazin-1-yl)-phenyl, 3-[4-(2-methoxy-ethyl)-piperazin-1-yl]-phenyl, 3-[4-(2-ethoxy-ethyl)-piperazin-1-yl]-phenyl, 3-(2-pyrrolidin-1-yl-ethoxy)-phenyl, 3-(2-dimethylamino-ethoxy)-4-methoxy-phenyl, 4-dimethylaminomethyl-phenyl, 4-(4-methyl-piperazin-1-ylmethyl)-phenyl, 4-[1-(2-methoxy-ethyl)-piperidin-4-ylmethyl]-phenyl, 4-methoxy-3-(2-piperidin-1-yl-ethoxy)-phenyl, 3-[4-(4-ethyl-piperazin-1-yl)-phenyl]-2,2-dimethyl-propionyl, 3-[4-(4-propyl-piperazin-1-yl)-phenyl]-propionyl, 3-(4-pyrrolidin-1-yl-phenyl)-propionyl, 3-[3-(4-ethyl-piperazin-1-yl)-phenyl]-2,2-dimethyl-propionyl, 3-{3-[4-(2-methoxy-ethyl)-piperazin-1-yl]-phenyl}-2,2-dimethyl-propionyl, 3-{3-[4-(2-ethoxy-ethyl)-piperazin-1-yl]-phenyl}-2,2-dimethyl-

propionyl, 3-(3-pyrrolidin-1-yl-phenyl)-propionyl, 2-[4-(4-methyl-piperazin-1-yl)-phenyl]-isobutyl, 2-(4-methoxy-phenyl)-acetyl, 2-(3-methoxy-phenyl)-acetyl, 2-[4-(4-methyl-piperazin-1-yl)-phenyl]-acetyl, 2-[4-(4-ethyl-piperazin-1-yl)-phenyl]-acetyl, 2-[4-(4-isopropyl-piperazin-1-yl)-phenyl]-acetyl, 2-(4-pyrrolidin-1-yl-phenyl)-acetyl, 2-[4-(2-diethylamino-ethylamino)-phenyl]-isobutyl, 2-(4-pyrrolidin-1-yl-phenyl)-isobutyl.

Particularly preferred compounds are examples as disclosed in WO 03/020278A1, pp. 17-52.

All the cat K inhibitors mentioned above as an alternative class of cat K compounds for use in the invention are known from the literature. This includes their production (see e.g. WO 03/020278A1, pp. 9-12).

A "cathepsin K inhibitor" is a compound that binds to and inhibits the function of cathepsin K in one or more cells or tissues. Cathepsin K is e.g. disclosed in Tetzuka et al., 1994, J Biol Chem 269: 1106-1109 and includes isoforms or mutations of it, and a protein having at least 95% homology to cathepsin K.

The term "effective amount" in connection with a cathepsin K inhibitor means an amount capable of treating a bone-resorbing disease, a neoplastic disease, arthritis, a disease exacerbated by the presence of cathepsin K inhibitors or a disease improved by the presence of cathepsin K inhibitors; activating the function of cathepsin K in a bone cell; inhibiting the function of cathepsin K in a cancer cell; inhibiting the expression of cathepsin K in a cell; or inhibiting the growth of a neoplastic cell.

The term "effective amount" in connection with another therapeutic agent means an amount capable of treating or preventing a bone-resorbing disease, a neoplastic disease, arthritis, a disease exacerbated by the presence of estrogen or a disease improved by the presence of cathepsin K inhibitors; activating the function of cathepsin K in a bone cell; inhibiting the function of cathepsin K in a cancer cell; inhibiting the expression of cathepsin K in a cell; or

inhibiting the growth of a neoplastic cell, while the cathepsin K inhibitor is exerting its  
therapeutic or prophylactic effect.

The term "a severe form of bone loss diseases" means one severe form of bone loss diseases as defined above or can mean several severe forms of bone loss diseases.

A "patient" is an animal, including, but not limited to, an animal such a cow, monkey, horse, sheep, pig, chicken, turkey, quail, cat, dog, mouse, rat, rabbit, and guinea pig, in one embodiment a mammal, in another embodiment a human.

The invention is further described by way of illustration in the following Examples.

EXAMPLEExample 1:

**Safety and efficacy of N-[1-(cyanomethyl-carbamoyl)-cyclohexyl]-4-(4-propyl-piperazin-1-yl)-benzamide (Compound A) on Bone Mineral Density (BMD), Bone Structure, and Bone Strength in Ovariectomized (OVX) Cynomolgus Monkeys after daily oral treatment for 18 months**

### 1.1. GROUPS AND STUDY DESIGN

One hundred (100) female cynomolgus monkeys (*Macaca fascicularis*) are dosed twice daily by oral gavage for (18) months. The in-vivo portion of the study take place at Shin Nippon Biomedical Laboratories, Ltd. in Kagoshima, Japan. Table I describes the treatment groups. Group assignment of animals are done based on body weight and the result of first baseline densitometry results (DXA and pQCT) in a way that equivalent values for all these 3 data variables are achieved. Prior to treatment initiation, (80) animals undergo bilateral ovariectomies and (20) animals undergo sham-ovariectomies. Duplicate DXA and pQCT measurements are performed at baseline (time 0), and single measurements thereafter at 3 months, 6 months, 9 months, 12 months, and 18 months of the dosing period ( $\pm$ 2 weeks for each timepoint). Serum and urine are collected on two occasions prior to dosing, and at 3 months, 6 months, 9 months, 12 months, and 18 months of the treatment period ( $\pm$ 2 weeks for each timepoint). Serum biochemistry are performed at SkeleTech, Inc. in Bothell, WA 98021, USA as described in Sections 4.4 of this protocol. The monkeys are necropsied after 18 months of dosing and select bones and soft tissues are collected. Bones are analysed for histology, histomorphometry, and biomechanical testing as described in sections 4.11, 4.12, and 4.13 of this protocol.

**Table 1: Grouping of Study Animals**

Group	OVX Status	TGSE Article	Dose Level (mg/kg/day)	Number of Animals
1	Sham	Vehicle	0	20
2	OVX	Vehicle	0	20

3	OVX	Compound A	Low	20
4	OVX	Compound A	Medium	20
5	OVX	Compound A	High	20

<sup>1</sup>Group 1 will consist of spayed-operated monkeys.

**Table 2: Experimental Evaluations**

	*						
OVX/Sham surgery	*						
DXA	* <sup>1</sup>	*	*	*	*	*	*
QCT	* <sup>1</sup>	*	*	*	*	*	*
Serum (BSALK, OSCL, SCRL, SNTX, EST, creatinine)	* <sup>1</sup>	*	*	*	*	*	*
Urine Collection	* <sup>1</sup>	*	*	*	*	*	*
Radiographs	*						*
Pharmacokinetic Study				*			
Fluorochrome Labeling							*
Necropsy							*

<sup>1</sup> DXA and QCT scans, serum and urine collection will be performed twice prior to treatment initiation.

This study is conducted in compliance with the United States Food and Drug Administration Good Laboratory Practices (GLP) for Non Clinical Laboratory Studies (21 CFR Part 58, 1994).

## 2. COMPOUNDS

### 2.1 Test compounds

Code Name:

Compound A = N-[1-(cyanomethyl-carbamoyl)-cyclohexyl]-4-(4-propyl-piperazin-1-yl)-benzamide  
(preparation disclosed in e.g. WO 01/58886)

Storage Conditions:

at -15°C for solid form, 4C for solutions

**2.2 Preparation and administration of test compounds**

Preparation Method: Dissolve compound in water (5 mg/mL)  
 Administration Route: Oral  
 Administration Method: Test compound will be administered orally by nasogastric intubation  
 Administration Frequency: Twice daily (tbd), 7 days a week  
 Administration Volume: 5 ml/kg  
 Administration Time: 7:00 - 9:00, 16:00 – 18:00  
 Test compound groups :  
     A) Compound A low dose tbd  
     B) Compound A middle dose tbd  
     C) Compound A high dose (5x middle dose) tbd

**3. STUDY ANIMALS****3.1 Type**

Species and Sex: Female cynomolgus monkeys (*Macaca fascicularis*); Purpose-bred; Chinese origin  
 Body Weight: ~3-5 kg (at the initiation of acclimation)  
 Age: ~10 - 13 years old  
 Number of Animals screened: 105 females  
 Number of Animals for Study: 100 females with closed growth plates and free of abnormalities in spine radiographs  
 Justification for Selection of Study Species: This species is chosen because skeletal function and reproductive physiology are comparable to human beings.

**3.2 Animal Husbandry**

Animal Testing Facility: SNBL Kagoshima Primate Facility  
 Temperature: ~20°C - 30°C  
 Humidity: ~30% - 80%  
 Frequency of Ventilation: Approximately 15 times/hour  
 Illumination: 12:12, 12 hours dark, 12 hours light cycle

Animal Cage and Size: Stainless steel, cages conformed to NIH Standards  
Food: Teklad Certified 25% Monkey Diet (W)  
Harlan Sprague Dawley, Inc., USA  
(approximately 108g/day), 15:00 – 09:00  
Water: ad libitum  
Room Sanitation: Washed daily with water  
Cage Sanitation: Soiled trays washed daily with water

### 3.3 Identification of Animals and Cages

Identification of Individual Animals: Tattooing on the chest  
Identification of Cages: Color-coded cage cards indicating study number,  
group number, dose level and animal number

### 3.4 Pre-dosing Health Evaluation

Animals are monitored daily for signs of ill health. All sick animals are referred to the responsible clinician for evaluation and/or treatment.

## **4. OBSERVATIONS AND EXPERIMENTAL PROCEDURES**

### 4.1 Analysis of test articles

Aliquots of each concentration are retained during the first week of dosing and during month 9 when PK is done.

### 4.2 Clinical signs and food consumption

All animals are observed during the dosing period prior to and immediately after dosing. All sick animals are referred to the responsible clinician for evaluation and/or treatment. Throughout the study food consumption are noted as part of routine health check.

### 4.3 Body weight

Body weight for each animal is measured at the start of the study, once a month until time point 3 months, and at 3 month intervals throughout the remainder of the study.

#### 4.4 Schedule of baseline procedures

Procedure	Begin	End
Initial baseline blood and urine collection	April 9, 2001	April 13, 2001
Perform duplicate scans (DXA, pQCT)	April 16, 2001	April 20, 2001
Second baseline blood and urine collection	April 23, 2001	April 27, 2001
OVX, Sham-OVX	April 30, 2001	May 11, 2001

#### 4.5 Ovariectomy (OVX)

Prior to initiation of treatment, animals in groups 2-5 undergo bilateral ovariectomy and monkeys in group 1 undergo sham surgery. Serum estradiol levels are measured prior to ovariectomy or sham surgery and approximately 3, 6, 9, 12, and 18 months thereafter to confirm the success of ovariectomy.

#### 4.6 Blood sampling

Frequency:

Blood samples are collected on two occasions prior to treatment initiation, and 3, 6, 9, 12, and 18 months thereafter ( $\pm 2$  weeks for each time point).

Timing:

Blood samples are collected prior to the administration of test compounds.

Number of Animals:

All animals ( $n=100$  per time point)

Blood Sampling Method:

Blood samples are drawn from the femoral vein on days when bone scanning is also taking place, blood is collected prior to bone scanning. After approximately 30 minutes of stabilization at room temperature, serum is obtained by centrifugation (3000 rotations per minute [r.p.m.], 15 minutes). Serum samples designated for estradiol are stored

**Storage and Shipment:**

in borosilicate glass tubes, and all other samples are stored in polypropylene micro centrifuge tubes.

**Experimental Evaluations:**

Serum is aliquoted according to Table 3. All samples are labeled at minimum with animal number, date collected, timepoint, and volume. Serum samples are stored at ~-70°C or below.

Estradiol (EST), bone-specific alkaline phosphatase (BSALK), osteocalcin (OSCL), serum c-linked telopeptide (SCRL), and serum n-linked telopeptide (SNTX) are analyzed at each timepoint.

**Table 3: Serum Aliquots (volumes in  $\mu$ l)**

Parameter	Baseline		Month 1		Month 3		Month 6		Month 9		Month 12		Month 18	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
SCRL	120	120	120	120	120	120	120	120	120	120	120	120	120	120
SNTX	100	100	100	100	100	100	100	100	100	100	100	100	100	100
BSALK	100	100	100	100	100	100	100	100	100	100	100	100	100	100
EST	500	500	500	500	500	500	500	500	500	500	500	500	500	500
OSCL	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Spare	5*300	5*300	5*300	5*300	5*300	5*300	5*300	5*300	5*300	5*300	5*300	5*300	5*300	5*300
Total Sera Volume	2420	2420	2420	2420	2420	2420	2420	2420	2420	2420	2420	2420	2420	2420
Total mL blood	5	5	5	5	5	5	5	5	5	5	5	5	5	5

**4.7 Urine sampling****Frequency:**

Urine samples are collected on two occasions prior to treatment initiation, and 3, 6, 9, 12, and 18 months thereafter ( $\pm 2$  weeks for each time point).

**Number of Animals:**

All animals ( $n=100$  per time point).

**Method:**

Urine are collected over a  $24 \pm 1$  hour period in a metabolism cage. Urine volume are recorded.

**Storage and Shipment:**

Four (4), 500  $\mu$ L aliquots from each sample are placed in

polypropylene tubes. Excess urine are discarded. All samples are labeled at a minimum with the animal number, 'U' to indicate Urine, date collected, time point and volume. All urine samples are stored at approximately -20°C.

#### 4.8 Densitometry (DXA and pQCT)

- Frequency: DXA of the lumbar spine and femur are performed in duplicate prior to treatment initiation and single scans are performed thereafter at 3, 6, 9, 12, and 18 months of treatment ( $\pm$  1 month for each time point). pQCT of the left femoral neck are performed in duplicate prior to treatment initiation and single scans are performed thereafter at 3, 6, 9, 12, and 18 months of treatment ( $\pm$  1 month for each time point).
- Number of Animals: All animals (n=100)
- Measurement Method: DXA measurements are performed using a Lunar DPX $\alpha$ . pQCT scans are performed with a Stratec XCT 3000;. Scans are performed while animals are under ketamine sedation (approximately 10mg/kg)

#### 4.9 X-Ray Examination

- Frequency: Spine radiographs are taken prior to OVX/Sham surgery, and again prior to necropsy with the animal placed in the lateral position.
- Number of Animals: All animals (n=100).
- Measurement Method: Radiographs are taken while the animal is under ketamine sedation.

#### 4.10 Pharmacokinetic Study

- Frequency: Once during the experiment prior to the 9 month time point
- Number of Animals: 60 (groups 3-5)
- Blood Sampling Times: 0, 15, 30, 60, 120, 240, 360, 480 minutes after the morning

Dose &amp; Collection Methods

**Control Plasma:**

dose and 16 hours after the afternoon dosing

**Shipment of samples**

femoral vein using EDTA tubes. Plasma (~ 200 microL) is obtained by centrifugation and immediately stored at -70°C. In addition to plasma from treated animals, a 5 mL sample of pooled control plasma is used for PK analytics (standard curve, etc.).

All samples are labeled at minimum with the animal ID, collection date, study time point and volume.

#### 4.11 Fluorochrome Labeling

Before collection of necropsy bone specimens, all animals receive bone-seeking fluorochrome labels. The label compound, dose and administration route is recorded in the study notebook. Labels are given on a 1-14-1-7 (1 day label, 14 days no label, 1 day label, 7 days no label, necropsy) schedule. Labels are given to ketamine sedated animals.

#### 4.12 Necropsy

**Time point:**

Monkeys are necropsied approximately 18 months after the start of treatment.

**Number of Animals:**

100

**Method:**

Monkeys are initially be sedated with ketamine for radiography and serum collection. Pentobarbital (approximately 26 mg/kg) i.v. bolus are used to euthanize animals. At a minimum, the following bones are collected: second lumbar vertebra (LV2), right femur and left radius (all fixed in 70% ethanol); lumbar vertebra 3 (LV3), left tibia, left humerus, and left femur (each individually wrapped in saline-soaked gauze and bagged in plastic before storage at approximately -20°C). Soft tissues (to be determined by Sponsor) are collected and fixed in 10% neutral buffered formalin (NBF).

4-33509P1

Storage: Ethanol-fixed bone specimens are stored at approximately -20°C.

#### 4.13 Bone Histology

Bone histology are performed. At a minimum, LV2 and left femur are submitted for histology. All specimens are processed for embedding in a methyl methacrylate-based plastic. LV2 and left femoral neck specimens are sectioned using a sledge microtome or rotary microtome at 5-10 $\mu$ m, and mounted unstained and stained for bright field, polarized light and epifluorescence microscopy. Left femoral midshaft specimens are embedded, cut with a diamond saw, ground to approximately 50  $\mu$ m thick and mounted for polarized light and epifluorescence microscopy.

#### 4.14 Histomorphometry

Histomorphometry are performed. Structural and dynamic histomorphometry are performed on LV2 (cancellous bone), right femoral neck (cancellous bone) and right femoral midshaft (cortical bone). Static histomorphometry are performed on LV2 and right femoral neck (cancellous bone). The standard Annual Society of Bone and Mineral Research (ASBMR) nomenclature is employed. See Tables IV and V for more details on histomorphometric measurements and derived variables.

Table IV: Histomorphometric Measurements<sup>a</sup>

Parameter / Units / Name		
<b>Structural (Unstained Sections)</b>		
T.Ar	mm <sup>2</sup>	Tissue area = bone area + marrow area (Ma.Ar)
B.Ar	mm <sup>2</sup>	Bone area
B.Pm	mm	Bone perimeter
<b>Dynamic (Unstained Sections)</b>		
sL.Le	mm	Single label length
dL.Le	mm	Double label length
Ir.L.Wi	$\mu$ m	Inter-label width
<b>Static (Stained Sections)</b>		
O.Pm	mm	Osteoid perimeter

E.Pm	mm	Eroded perimeter
O.Wi	μm	Osteoid width
W.Wi	μm	Wall width

<sup>a</sup> Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RM. 1987. Bone histomorphometry: standardization of nomenclature, symbols, and units. J Bone Miner Res 2:595-610.

Table V: Derived Histomorphometric Variables<sup>a</sup>

Parameter			Equation
<b>Structural</b>			
BV/TV	%	Bone volume	$100 * B.Ar / T.Ar$
BS/BV	mm/mm <sup>2</sup>	Bone surface/volume	$(4/\pi) * B.Pm / B.Ar$
Tb.Th	μm	Trabecular thickness	$2000 / (BS/BV)$
Tb.N	/mm	Trabecular number	$(2/\pi) * B.Pm / T.Ar$
Tb.Sp	μm	Trabecular separation	$(1000 / Tb.N) - Tb.Th$
<b>Dynamic</b>			
sL.S/BS	%	Single label surface	$100 * sL.Pm / B.Pm$
dL.S/BS	%	Double label surface	$100 * dL.Pm / B.Pm$
MS/BS	%	Mineralizing surface	$100 * (L.Le / 2) / B.Pm$
MAR	μm/day	Mineral apposition rate	$(Ir.L.Wi * \pi / 4) / Ir.L.t$
BFR/BS	μm <sup>3</sup> /μm <sup>2</sup> /year	Bone formation rate, surface referent	$3.65 * MAR * MS / BS$
BFR/BV	%/year	Bone formation rate, bone referent	$0.1 * BFR / BS * BS / BV$
BFR/TV	%/year	Bone formation rate, bone referent	$0.01 * BFR / BV * BV / TV$
<b>Static</b>			
OS/BS	%	Osteoid surface	$100 * O.Pm / B.Pm$
ES/BS	%	Eroded surface	$100 * (E.Pm + Oc.Pm) / B.Pm$
Oc.S/BS	%	Osteoclast surface	$100 * Oc.Pm / B.Pm$

O.Th	$\mu\text{m}$	Osteoid thickness	$O.Wi^*\pi/4$
W.Th	$\mu\text{m}$	Wall thickness	$W.Wi^*\pi/4$
<b>Combined</b>			
Ac.f	/year	Activation frequency	$3.65*(OS/BS)/FP$
Aj.AR	$\mu\text{m}/\text{day}$	Adjusted apposition rate	$MAR^*(MS/BS)/(OS/BS)$
FP	Days	Formation period	$W.Th/Aj.AR$
It.Th	$\mu\text{m}$	Interstitial thickness	$Tb.Th - (2^*W.Th)$
Rs.P+Rv.P	Days	Resorption + reversal period	$FP^*(ES/BS)/(OS/BS)$

<sup>a</sup> Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RM. 1987. Bone histomorphometry: standardization of nomenclature, symbols, and units. J Bone Miner Res 2:595-610.

*Structural evaluation* of all sites include measurement of bone areas, marrow or tissue areas, and bone perimeters. Derived variables are calculated as indicated in the above table.

*Dynamic analysis* of all sites include measurement of inter-label width using a random sampling method, and measurement of total label length. Derived variables are calculated as indicated in the above table.

*Static (surface histological) analysis* of cancellous sites include measurement of osteoid, eroded and osteoclastic perimeters, osteoid width, and wall width. Derived variables for the static analysis and combined static and dynamic analyses are calculated as indicated in the above table.

#### 4.15 Biomechanics

Using an Instron Material Testing Machine (Model 4501 retrofit to a specification of Model 5500), three types of mechanical tests are performed on the LV3 lumbar vertebral body, the femur and tibia.

*Compression test of lumbar vertebrae.* Excised lumbar vertebrae have their two periphyseal ends, posterior pedicle arch and spinous process removed to provide an approximately 8 mm long section of the vertebral body, using a low speed saw. The volume of the vertebral body cylinder

are estimated by weighing the bone specimens before and during immersion in water, and the average gross-sectional area of each specimen are obtained by dividing the volume by the height. The vertebral body cylinder is tested along the cranial-caudal axis at a constant deformation rate of 2 mm/min. During compression, load-deformation curves are recorded and subsequently analyzed using the manufacturer's software (Merlin version II) for calculation of breaking load, stress (maximal stress on the stress-strain curve, breaking load adjusted for testing area), Young's modulus (maximum slope of the stress-strain curve, intrinsic hardness) and energy absorbed (area under the curve for load-deformation, overall bone strength).

*Compression test of femoral neck.* The left proximal femur are mounted in a fixation device and placed in the material testing machine. A vertical load conducted by a brass cylinder is applied to the top of the femoral head. The cylinder is directed parallel to the axis of the femoral diaphysis and moved at a constant rate of 2 mm/min until fracture of the femoral neck. The load-deformation is recorded and breaking load value obtained. The length of the long axis is measured on the bone or on a radiograph and the sectional moment of inertia in the femoral neck is obtained from the *in vivo* pQCT scan data.

*Three point bending test of long bones.* The femur and tibia shafts are placed in a compact 3 point flexure fixture. The distance between the supporting rods is fixed at 35 mm. Load is applied at a constant deformation rate of 2 mm/min with a rod at the midpoint. Load-deformation curves are recorded. After biomechanical testing, the axial moment of inertia, average cortical thickness, and medial-lateral and anterior-posterior diameters are collected by pQCT and caliper measurement of the reconstructed shaft specimen, or from contralateral histomorphometry data. Using the combinations of values obtained, breaking load, stress and Young's modulus are calculated.

#### 4.16 Laboratory analysis

Serum biomarkers: The serum bone resorption markers crosslinked N-telopeptide (NTx, Ostex, order no. 9021) and CrossLaps (SCRL, Osteometer, order no. 4CRL4000) are assayed with enzyme-linked immunosorbent assays (ELISA). The bone formation markers osteocalcin (ELISA, Metra BioSystems, #8002) and bone-specific alkaline phosphatase (ELISA, Metra BioSystems, #8012) are assayed.

Serum hormones: Serum estradiol are assayed using RIA from DPC , order no. KE2D1.

Blood chemistry: Serum creatinine (Roche, 47003) are assayed on a Cobas MIRA.

## 5. DATA ANALYSIS

All data are first be checked to ensure they meet the assumptions for parametric analysis (normality, homogeneity of variances). Data is transformed if necessary to meet the assumptions.

In general, data is analyzed by one-way analysis of variance for variables assessed only once during the treatment phase of the study. For variables assessed repeatedly during the treatment phase, a two-way (group, time) analysis of variance with repeated measures on time is used. For both one-way and two-way analyses, if pretreatment values are available they are used as covariates to create an analysis of covariance. Significant group effects in these analyses are evaluated using an appropriate correction (for example, Dunnett's) in which the type I error rate is corrected to ensure a 5% error rate. Differences between groups are compared to group 2 (Ovx). Additional analyses may be employed as indicated by the structure of individual data sets and results of these basic analyses. Results are presented in tabular form as Mean  $\pm$  SEM (standard error of mean), and if useful to visualize the data, graphically. Overall significance values as well as specific group comparisons are presented in the tables.

## 6. RESULTS

- 1.) OVX (ovarectomized monkeys) increased bone turnover as shown in other primate studies. Compound A causes a dose-dependent reduction of resorption and formation markers. CrossLaps is reduced for 9 months, whereas NTx, the marker exclusively generated by cathepsin K, is reduced to sham by the mid dose and below sham by the high dose of compound A throughout the 18 month period. Formation markers are, however, only partially inhibited by compound A. In contrast, the bisphosphonates alendronate and zoledronic inhibit formation markers to sham or even below sham.
- 2.) Vertebral BMD and BMC increased in sham animals due to weight increase. OVX prevents the BMD increase in the spine and decreases femoral BMD and BMC, and compound A prevents the effect of OVX on lumbar spine BMD at all 3 doses. At the femur, compound A not only prevents loss of BMD by OVX, but BMD is increased above sham at all sites as measured by DXA . Whole femur BMC is also increased above sham

by compound A. Measured by pQCT, compound A prevents the effect of OVX on BMD

at all sites tested, compound A is at least as effective as bisphosphonates in preserving bone mass.

- 3.) OVX led to decreases in bone strength although the differences are not significant for many parameters analyzed. Compound A increases lumbar vertebrae strength in the compression test at the mid and high dose significantly. Strength and BMD are significantly correlated in all groups indicating normal bone quality in Compound A-treated animals. Compound A also prevents the non-significant decrease of femoral midshaft strength (3-point-bending test). The decrease in femoral neck stiffness is prevented by compound A. Effects on femoral neck strength does not reach significance, as has been observed in monkey studies with all other compounds (bisphosphonates, estrogen, SERM's).
- 4.) Compound A reduces bone turnover dose-dependently in cancellous bone at the mid and high dose, as would other anti-resorptive agents like bisphosphonates do. In addition, compound A decreases endocortical (transitional zone) bone turnover in vertebra, again in line with an anti-resorptive action. However, compound A stimulates periosteal bone formation in vertebra and mid-femur, and endocortical bone formation in the mid-femur, which leads to an increasing cortical thickness (as does PTH). The low dose stimulates periosteal bone formation without inhibiting cancellous bone turnover, which is important for the understanding of the effects of the low dose group on BMD. In addition, compound A promotes cortical thickness without increasing cortical porosity (in contrast to PTH), which is a desirable effect.
- 5.) In summary, compound A acts as expected as an antiresorptive agent in the cancellous and endocortical bone compartment in vertebrae and long bones, and surprisingly stimulates cortical bone formation at the subperiosteal site (again in vertebrae and long bones) like a bone forming agent would do.

#### Example 2: Clinical Study with Compound A

This is a 12-week treatment, multicenter, double-blind, randomized, placebo-controlled, parallel

group, dose-ranging, safety, tolerability and efficacy trial with Compound A in postmenopausal women, with 3 weeks follow-up.

The primary objectives of the study are to assess the effect of Compound A on biochemical markers of bone resorption and bone formation, and to evaluate its safety and tolerability profile. Secondary objectives are to assess the changes in biochemical markers after the end of treatment, and to study the pharmacokinetics of Compound A and one of its metabolite during and after 12 weeks of treatment.

The population of subjects is normal healthy postmenopausal women, i.e. paid volunteers. The reason for not investigating osteopenic women is the following: The efficacy endpoints of the study are biochemical markers of bone turnover (CTX, NTX, serum osteocalcin, BSAP). These variables are not directly correlated with BMD in man. Therefore we do not need to assess BMD and can include normal postmenopausal women. They are at least 5 years postmenopause, mainly because biomarkers are expected to fluctuate less in these women than in the perimenopause. Since the subjects included in the trial will not have any benefit whatsoever, and since the trial is rather demanding with a large number of assessments, including occult blood in stool, and PK, it is also ethically correct to include paid volunteers.

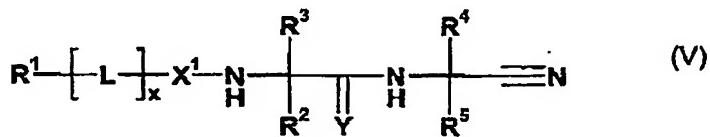
Four doses are tested, 5, 10, 25, and 50 mg od. The duration of the study is 12 weeks, with a 3-week follow-up. 12 weeks treatment allows to assess the time-course of biomarkers of both, bone resorption and bone formation and to ascertain that a steady-state in biomarkers is achieved.

#### Results:

- Compared to placebo (week 12), all doses had a greater reduction in serum CTX
- Dose-response relationship vs. placebo at all time points
- All dose groups, statistically significant at 10 hours after first study drug intake (only measurement taken at this time; may be influenced by circadian variation)
- Results from other resorption biomarkers (serum NTX, urinary NTX) support results seen with serum CTX
- For bone formation markers (serum osteocalcin, BSAP) over time, the decrease of suppression is less than that seen with the bone resorption markers

## **CLAIMS**

1. A method for the treatment of a severe form of bone loss diseases in a patient in need of such treatment which comprises administering an effective amount of a cathepsin K inhibitor to the patient.
  2. The use of a cathepsin K inhibitor in the preparation of a medicament for the treatment of a severe form of bone loss diseases.
  3. A pharmaceutical composition which incorporates as an active agent a cathepsin K inhibitor for use in the treatment of a severe form of bone loss diseases.
  4. A method, use or composition according to any preceding claims, wherein the cathepsin K inhibitors are used to stimulate bone growth in a patient in need of such a treatment.
  6. A method, use or composition according to any preceding claims, wherein the diseases are a severe form of osteoporosis, rheumatoid arthritis, osteoarthritis and tumour formation.
  7. A method, use or composition according to any preceding claims, in which the cathepsin K is selected from the following compounds of formula V or a pharmaceutically acceptable salt thereof, or any hydrate thereof



wherein

$\text{R}^1$  is optionally substituted (aryl, aryl-lower alkyl, lower alkenyl, lower alkynyl, heterocyclyl or heterocyclyl-lower alkyl);

$R^2$  and  $R^3$  together represent lower alkylene, optionally interrupted by O, S or  $NR^6$ , so as to form a ring with the carbon atom to which they are attached, and  $R^6$  is hydrogen, lower

alkyl or aryl-lower alkyl;

$R^4$  and  $R^5$  are independently H, or optionally substituted (lower alkyl or aryl-lower alkyl),  $-C(O)OR^7$ , or  $-C(O)NR^7R^8$ , wherein  $R^7$  is optionally substituted (lower alkyl, aryl, aryl-lower alkyl, cycloalkyl, bicycloalkyl, bicycloalkyl or heterocyclyl), and  $R^8$  is H, or optionally substituted (lower alkyl, aryl, aryl-lower alkyl, cycloalkyl, bicycloalkyl, bicycloalkyl or heterocyclyl); or

$R^4$  and  $R^5$  together represent lower alkylene, optionally interrupted by O, S or NR<sup>6</sup>, so as to form a ring with the carbon atom to which they are attached, and R<sup>6</sup> is hydrogen, lower alkyl or aryl-lower alkyl; or

$R^4$  is H or optionally substituted lower alkyl and  $R^5$  is a substituent of formula  $-X^2-(Y^1)_n-(Ar)_p-Q-Z$  wherein

$Y^1$  is O, S, SO, SO<sub>2</sub>, N(R<sup>6</sup>)SO<sub>2</sub>, N-R<sup>6</sup>, SO<sub>2</sub>NR<sup>6</sup>, CONR<sup>6</sup> or NR<sup>6</sup>CO;

N is zero or one;

P is zero or one;

$X^2$  is lower alkylene; or when n is zero,  $X^2$  is also C<sub>2</sub>-C<sub>7</sub>-alkylene interrupted by O, S, SO, SO<sub>2</sub>, NR<sup>6</sup>, SO<sub>2</sub>NR<sup>6</sup>, CONR<sup>6</sup> or NR<sup>6</sup>CO; and R<sup>6</sup> is hydrogen, lower alkyl or aryl-lower alkyl;

Ar is arylene;

Z is hydroxyl, acyloxy, carboxyl, esterified carboxyl, amidated carboxyl, aminosulfonyl, (lower alkyl or aryl-lower alkyl)aminosulfonyl, or (lower alkyl or aryl-lower alkyl)sulfonylaminocarbonyl; or Z is tetrazolyl, triazolyl or imidazolyl;

Q is a direct bond, lower alkylene, Y<sup>1</sup>-lower alkylene or C<sub>2</sub>-C<sub>7</sub>-alkylene interrupted by Y<sup>1</sup>; X<sup>1</sup> is  $-C(O)-$ ,  $-C(S)-$ ,  $-S(O)-$ ,  $-S(O)_2-$ , or  $-P(O)(OR^6)-$ , and R<sup>6</sup> is as defined above;

Y is oxygen or sulphur;

L is optionally substituted -Het-, -Het-CH<sub>2</sub>- or -CH<sub>2</sub>-Het-, and Het is a hetero atom selected from O, N or S; and

X is zero or one; and

aryl in the above definitions represents carbocyclic or heterocyclic aryl.

8. A method, use or composition according to any preceding claims, in which the cathepsin K inhibitor is N-[1-(cyanomethyl-carbamoyl)-cyclohexyl]-4-(4-propyl-piperazin-1-yl)-benzamide, or a pharmaceutically acceptable salt thereof, or any hydrate thereof.

9. All novel compounds, processes, methods and uses substantially as hereinbefore described  
with particular reference to the Examples

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